Northeastern CAT

SAC Crystallography Crosscut Review ‘07

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National Center for Research Resources

Mission Statement:

Design, construct and operate synchrotron beamlines for technically challenging problems in structural biology using the APS Tandem Offset Undulator (TOU) and a standard sector dipole source.

Microdiffraction Hardware and Software for Tough Cases

Funded through a combination of Funds from our member institutions and a major grant from the NIH's National Center for Research Resources (P41).

50% share of Operational days to Institutional Members
50% share to our NCRR collaborators and APS General Users

Classes of Challenging Samples

Microscopic Crystals

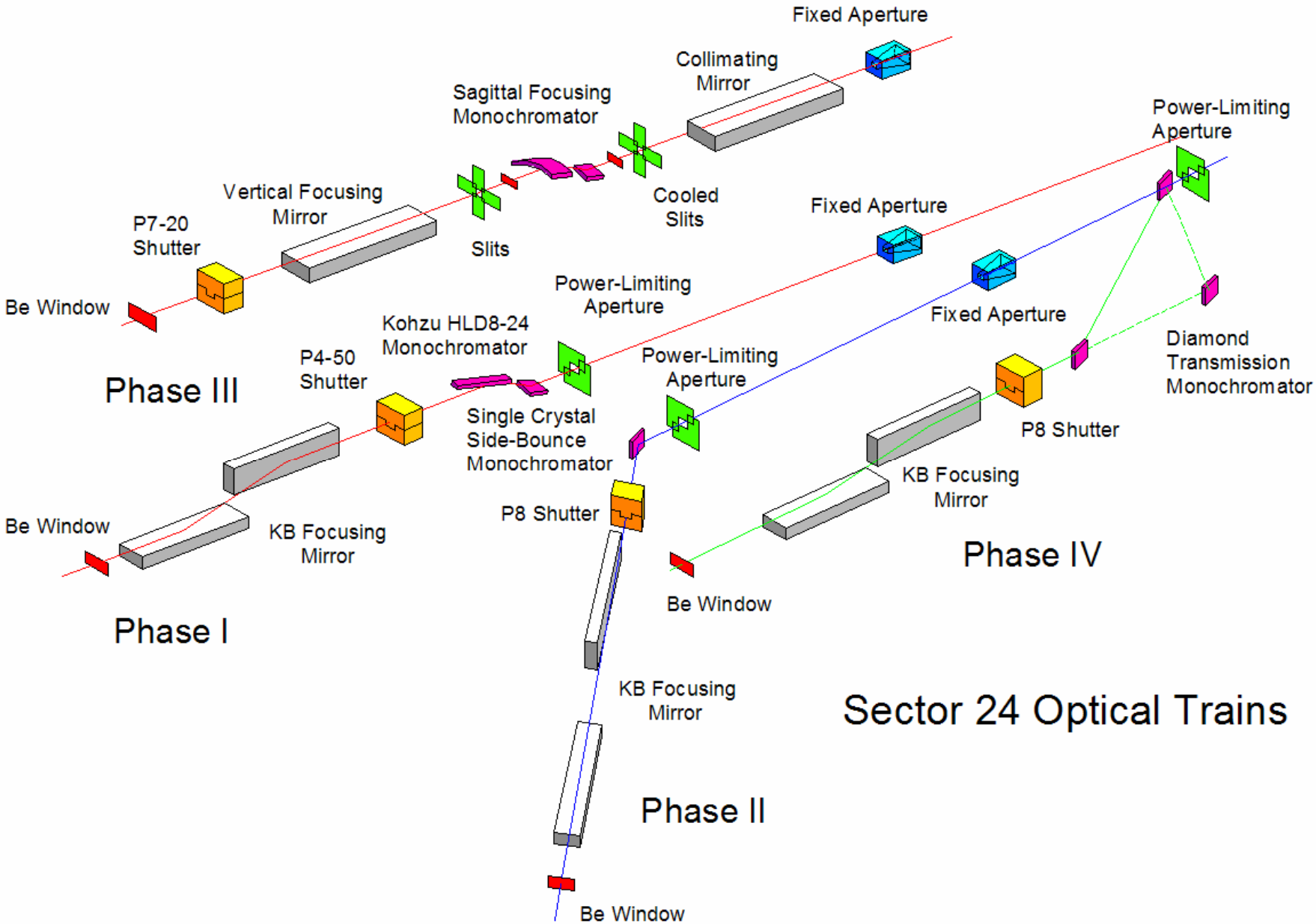
Weak Diffractors

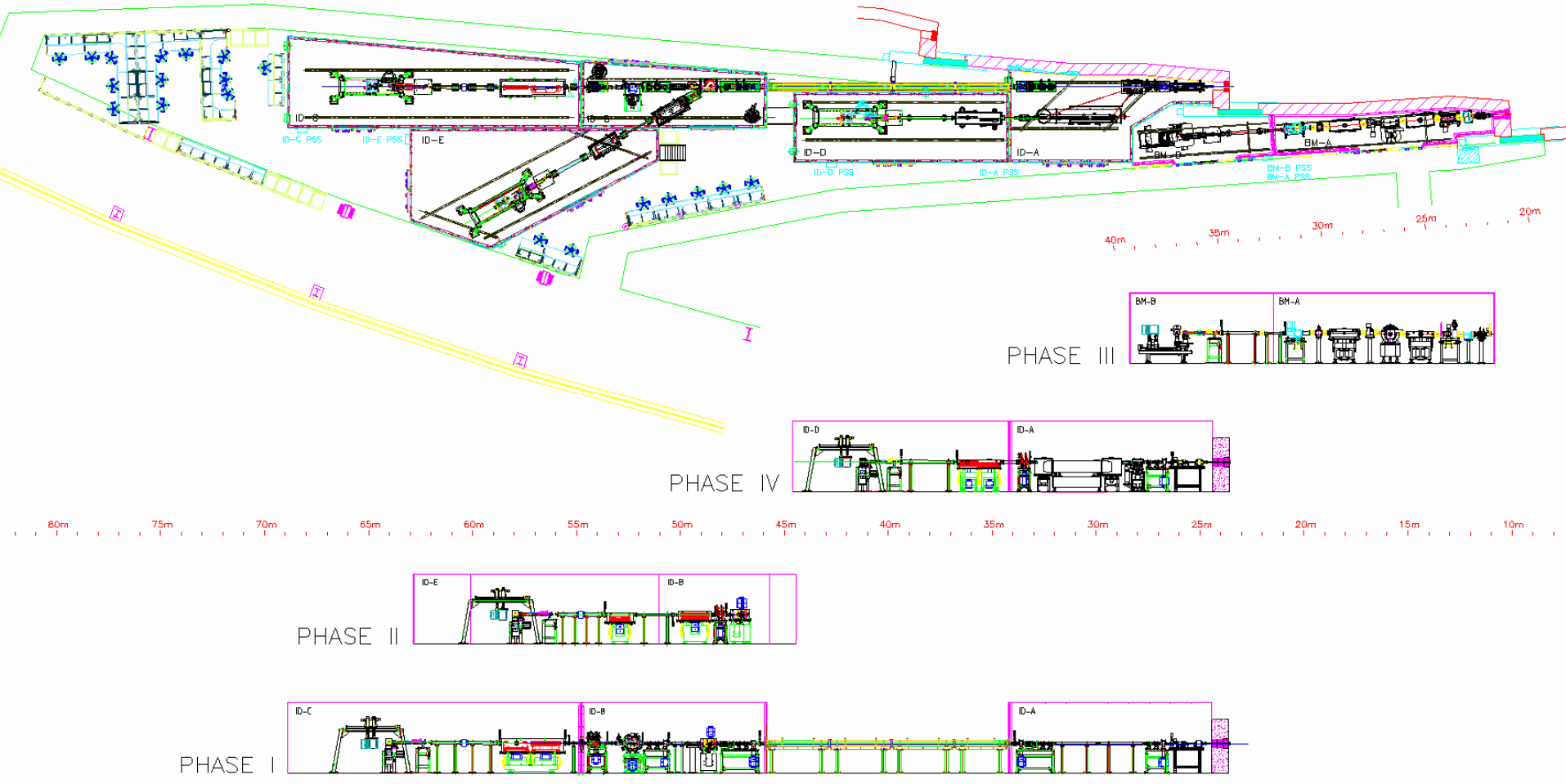
Large Unit Cells

- Viruses - Steve Harrison: Herpes, Rotovirus,
- Multisubunit complexes Molecular Machines with little internal symmetry
  - Jamie Cate: 70S ribosome
  - Tom Steitz 50S ribosome, DNA synthetases

Large Scale Projects

- Cases requiring large scale screening
  - Tom Schwartz, Gunter Blobel: nuclear pore complex
  - Nikola Pavletich: Kinases
- Functional Studies of Complex Molecular Machines - ribosomes
- Longitudinal Studies, e.g. Pathways/Functional Networks
  - Steve Ealick: Nucleotide Metabolism


Fixed Aperture
Collimating Mirror
Sagittal Focusing Monochromator
Power-Limiting Aperture
Vertical Focusing Mirror
P7-20 Shutter
Slits
Cooled Slits
Fixed Aperture
Be Window
Power-Limiting Aperture
Kohzu HLD8-24 Monochromator
Fixed Aperture
Diamond Transmission Monochromator
P4-50 Shutter
Power-Limiting Aperture
Phase III
Single Crystal Side-Bounce Monochromator
P8 Shutter
KB Focusing Mirror
Be Window
P8 Shutter
KB Focusing Mirror
Phase IV
Be Window
Phase I
KB Focusing Mirror
Sector 24 Optical Trains
Phase II
Be Window

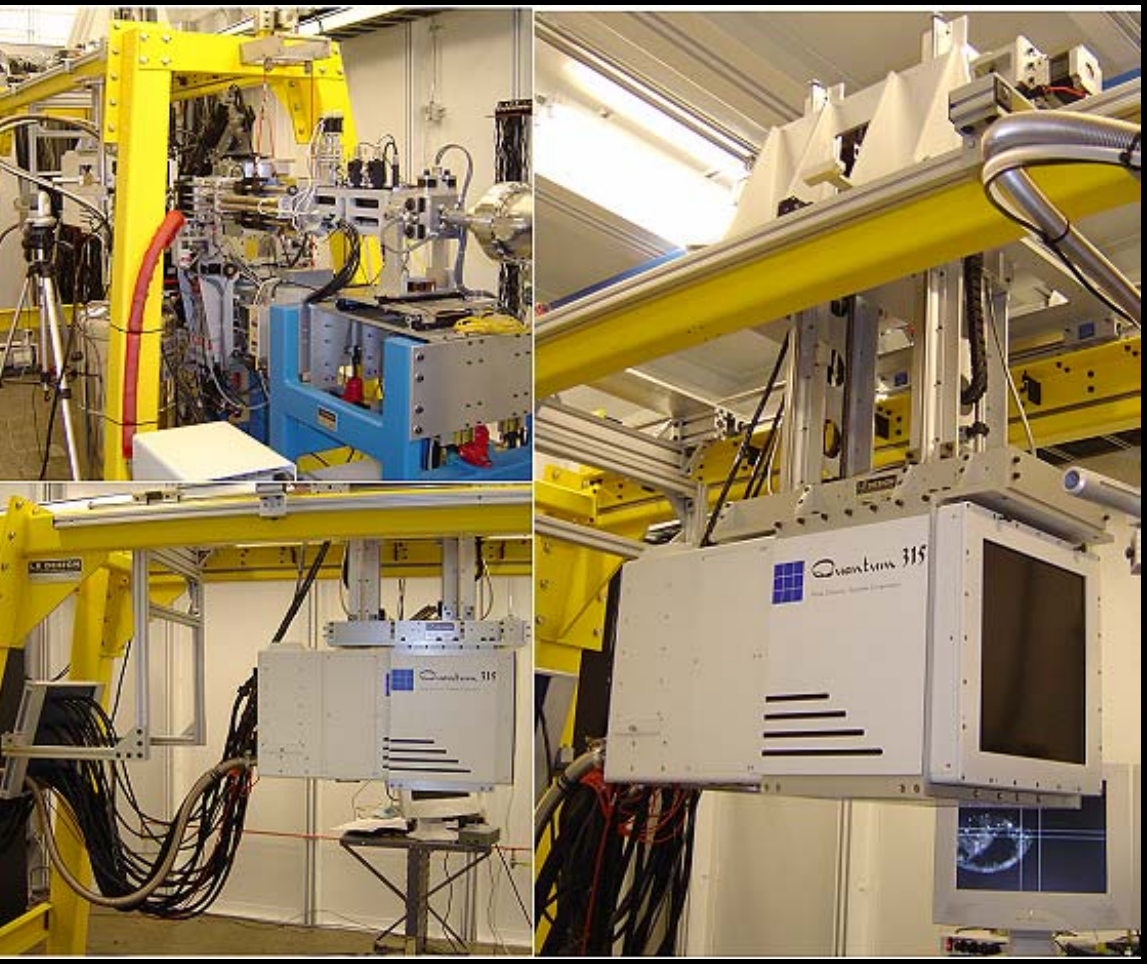


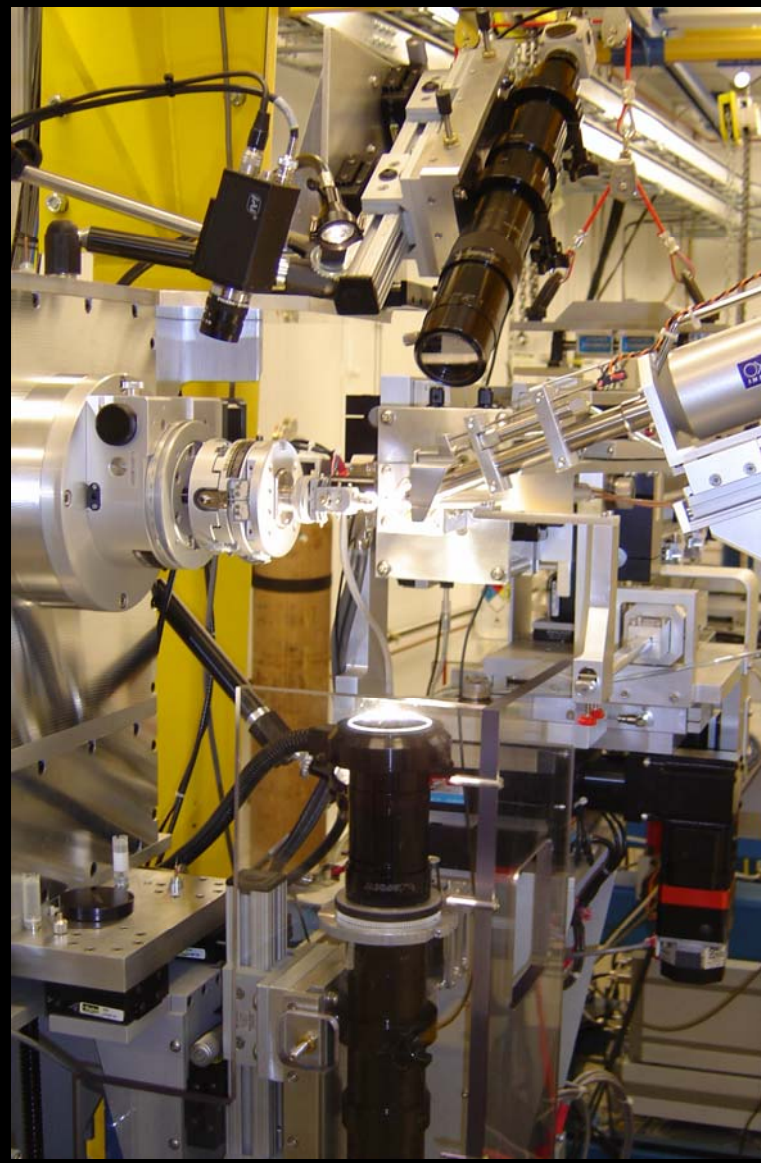
PHASE III

PHASE IV

PHASE II

PHASE I


Quantum 315



NE-CAT is specifically organized to address "challenging" data issues

1st Organizational principal is doctrinal:

NE-CAT beam lines are considered by staff and users to be reconfigurable, adaptable systems – even to the extent that this policy interferes with conventional productivity measures.

Hardware and Sofware issues

NE-CAT users are expected to collaborate with NECAT staff to define and evolve operational and technical range/scope of beam lines.

2nd Organizational principal: scale

NE-CAT must have sufficient fiscal and manpower resources to evolve and adapt Sector 24 beam lines in response to changing user requirements

NE-CAT will have 4 beam lines with disparate operational envelopes.

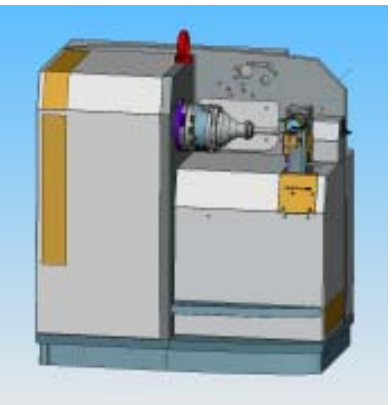
a) Therefore NE-CAT can realistically contemplate evolution (continuous development) of beam lines without incapacitating the entire operation.

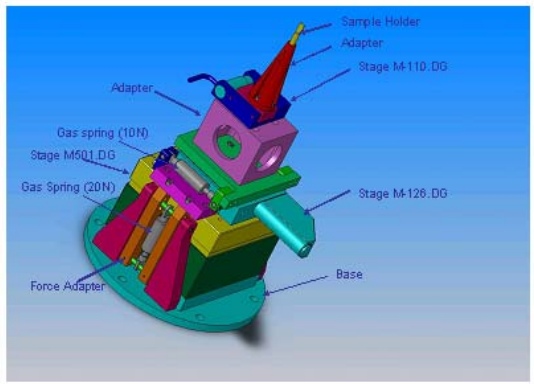
b) Once all beam lines are operational 24-ID-C can be evolved to satisfy changing user requirements.

c) "Standard" or less demanding studies go to one of the other 3 beam lines.

Ongoing / Future Beam Line Developments

Microdiffraction – Support for micro crystals





Integration of MD2 microdiffractometer with 24-ID-E

Upgrade of 24-ID-C to microdiffraction capability

Logistical improvements – Support for all problem classes

Robotic sample loader

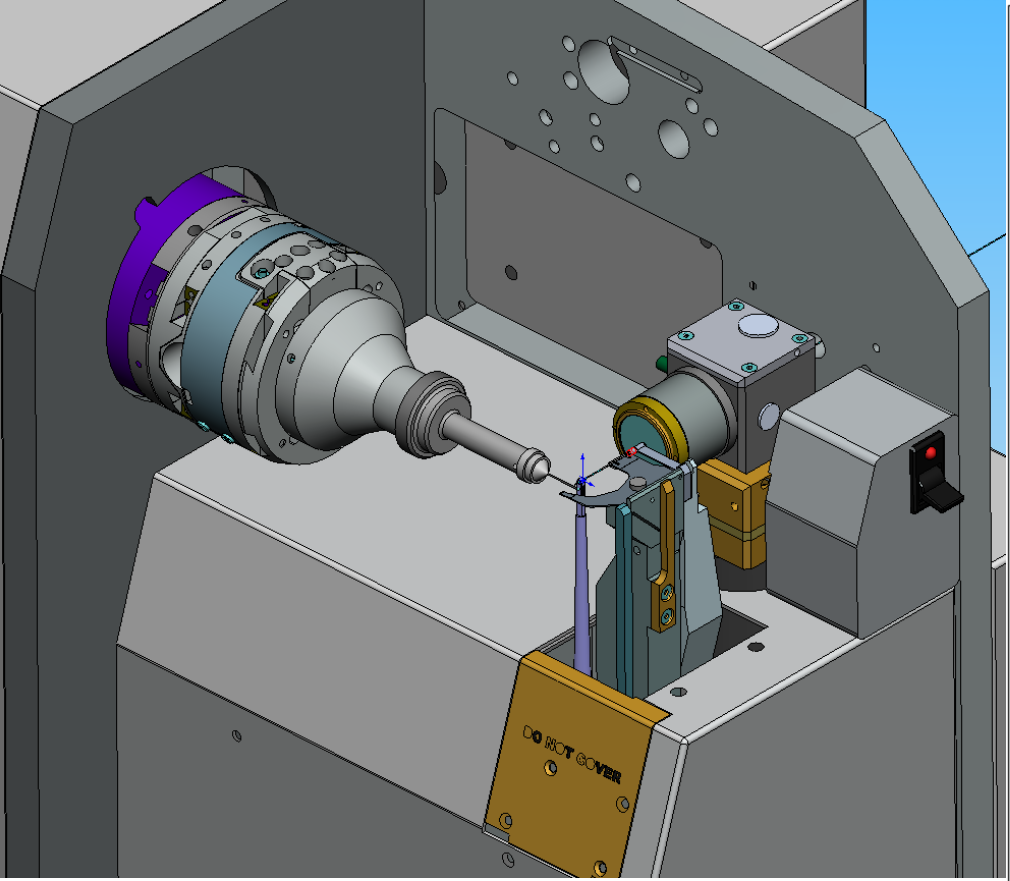
- reduce number of hutch entries

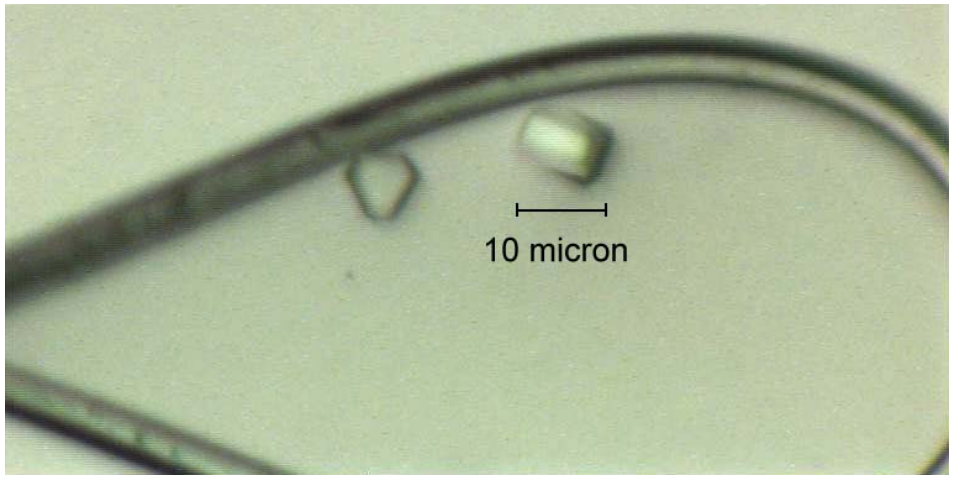
3.0 period undulators

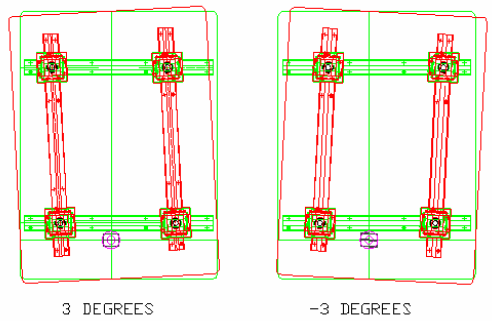
- avoid 1st to 3rd harmonic transistions

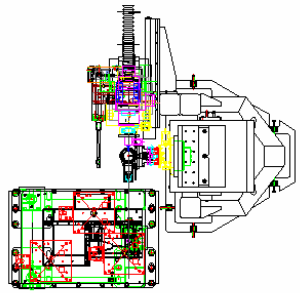
Clustered Computational Resource

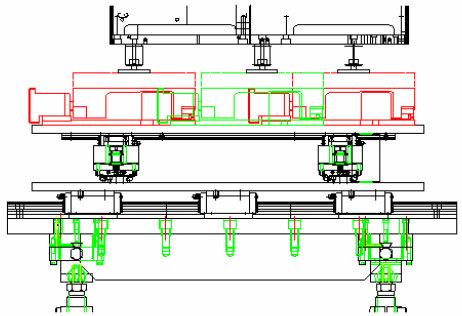
Telepresence for improved collaboration

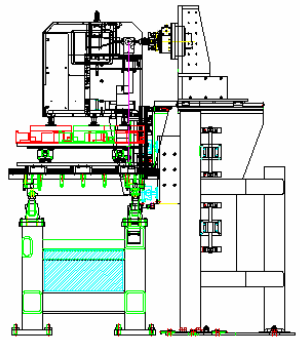

DO NOT COVER

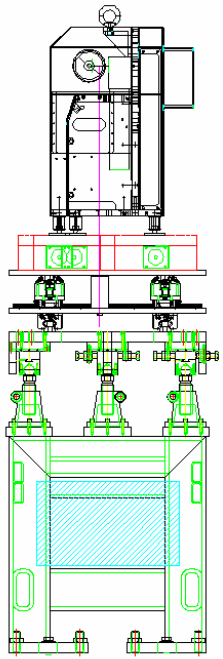

10 micron

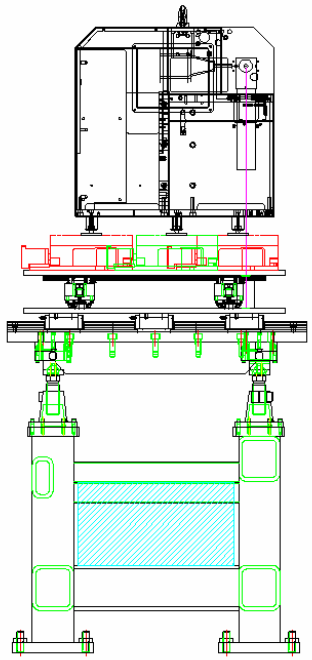

3 DEGREES
-3 DEGREES





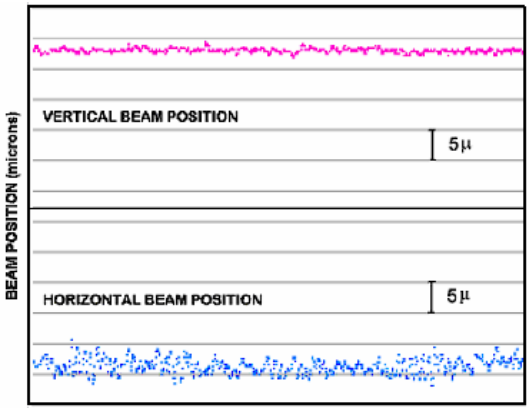






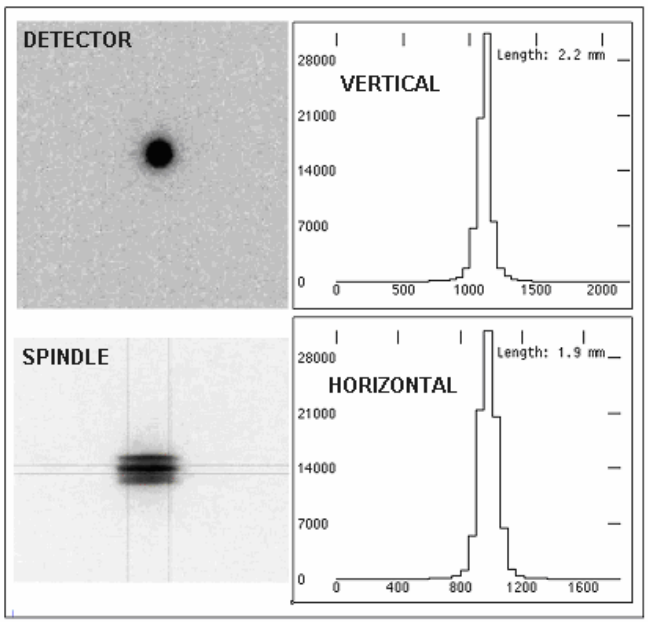
Ongoing / Future Beam Line Developments

Beam Positional Stability – Support for all problem classes

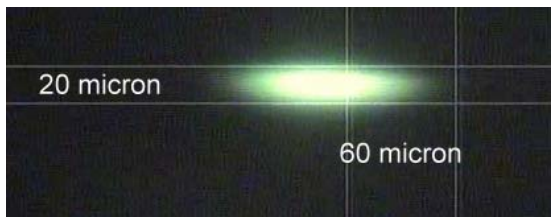


- Elimination of residual flow-induced vibrations
- Long baseline white BPM's to improve stability of white beams
- Improve mirror steering

Beam Focus – Support for all problem classes



- Compound Refractive Collimator
  - Increase Flux density
  - Reduce Horizontal Focus without Increasing demagnification
  - Reduce horizontal divergence without sacrificing focus
- 24-ID-C VFM converted to bimorph
  - Improved beam uniformity for over/under-focused setups



Crystal parameters:

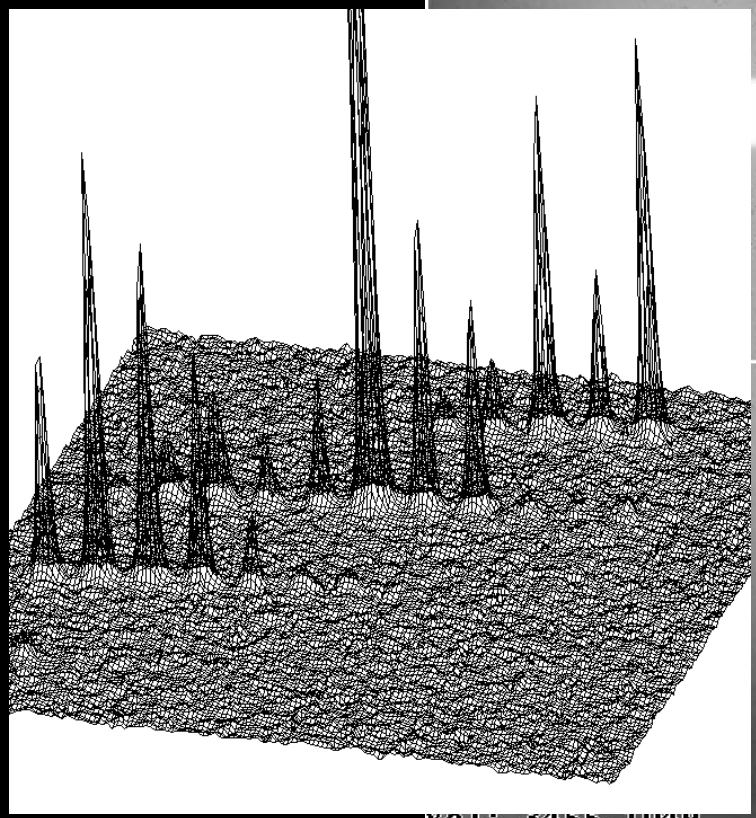
Space Group: $P2_12_12_1$

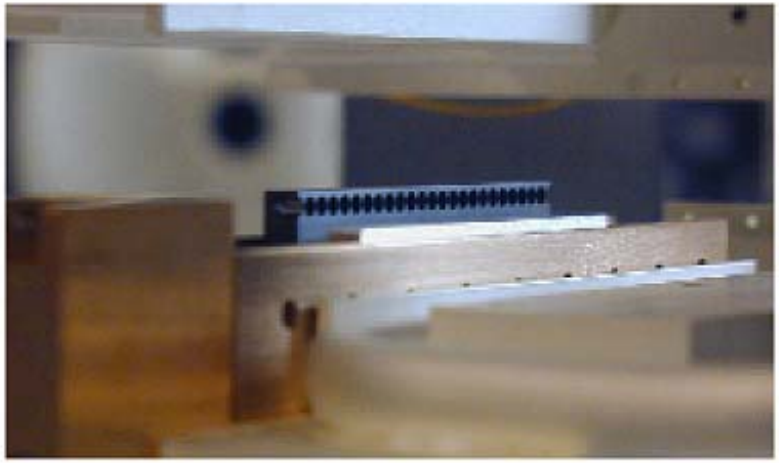
a = 740.75
b = 1198.07
c = 1345.41

one full ICP/ASU = 58 MD

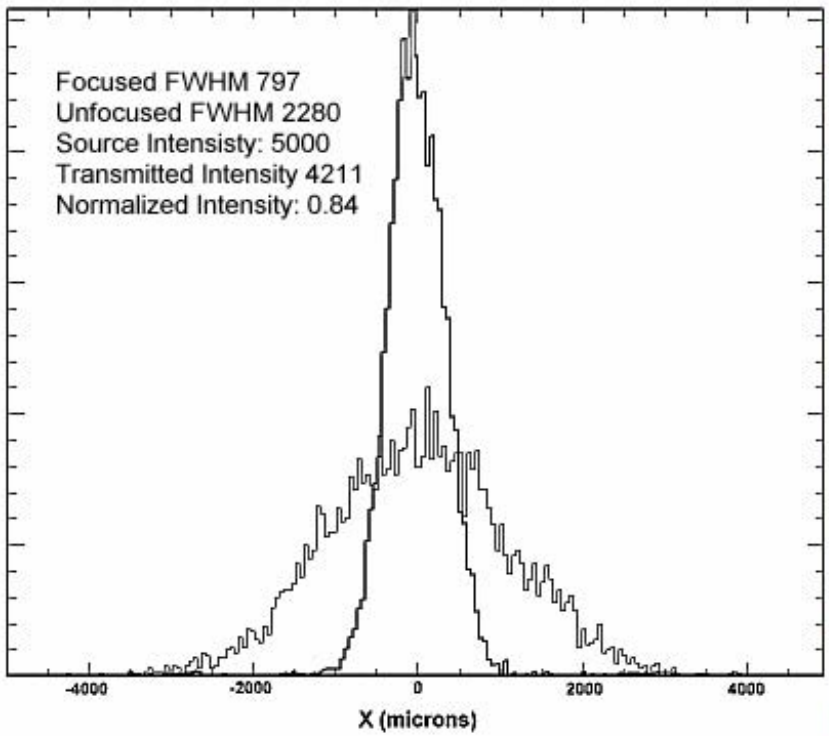
DLP virus particle ~600 A in diameter

Detector dist = 750 mm





Compound Refractive Collimating Lens



User Program / Productivity

Internal user program initiated mid 2005

Number of scientists visiting 24ID-C during 2006: 171

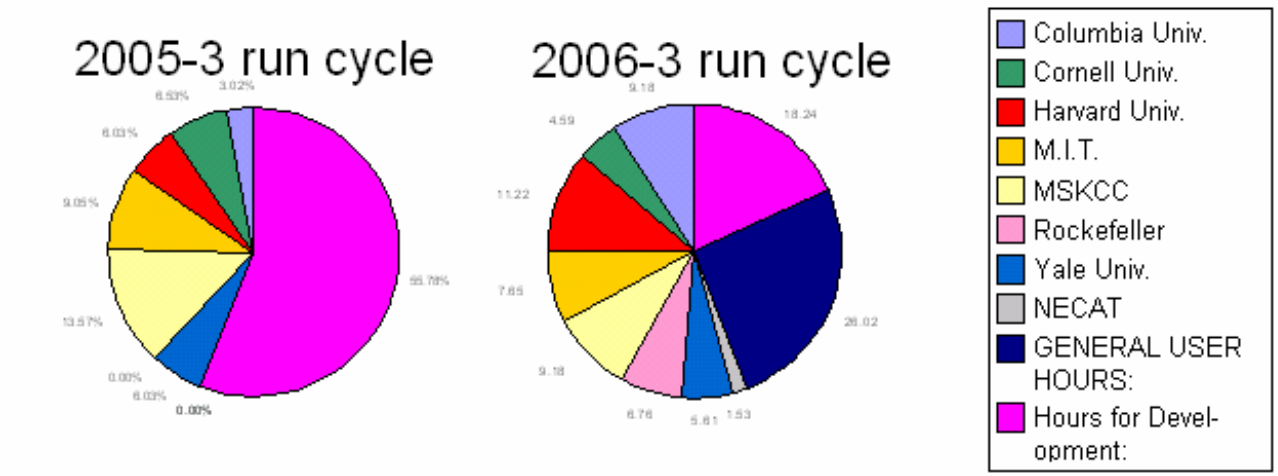
PDB submissions since late 2005: 57

Publications during 2006: 35

Cell: 3 Science: 3 Nature: 1 NSMB: 4 Mol Cell: 2 PNAS: 2

24ID-C entered APS GUP in 2006-3 run cycle

24ID-C User statistics

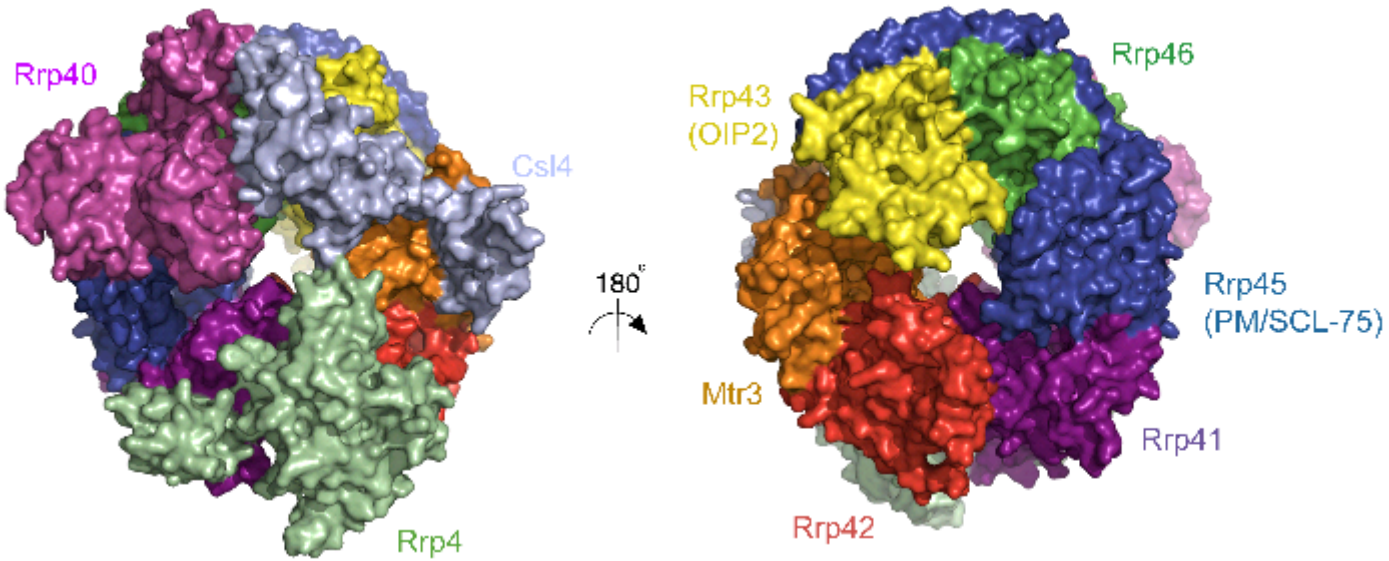


Science Highlights

Crystal Structure of the Eukaryotic RNA Exosome at 3.35Å

Q. Liu, J.C. Greimann and C.D. Lima, Cell, 127, 2006, 1223-1237

- Degradation and processing of cellular RNA (rRNA, snoRNA, snRNA)
- A 286kDa Reconstituted complex of nine proteins
- Cubic space group with cell edge of 308Å
- Radiation sensitive, hence a de-focussed beam was used to illuminate the whole ~200μ sized crystals

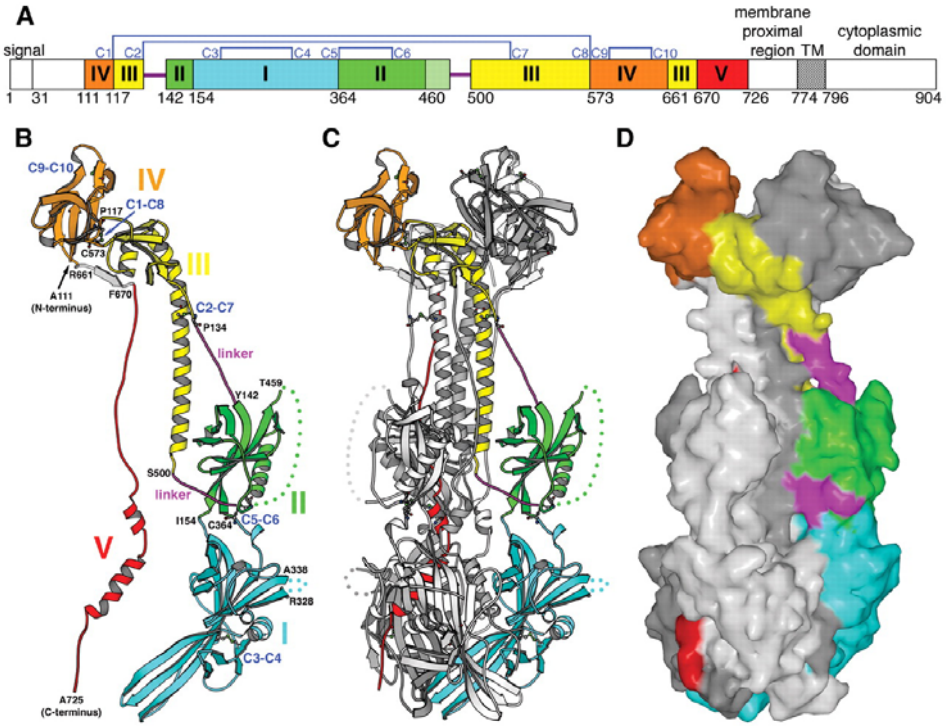


Two views of RNA Exosome

Science Highlights

Crystal Structure of Glycoprotein B from Herpes Simplex Virus 1 at 2.1Å
E.E. Heldwein, H. Lou, F.C. Bender, G.H. Cohen, R.J. Eisenberg, S.C. Harrison, Science, 313, 2006, 217 - 220

- Part of Herpes Simplex Virus cell entry complex.
- A trimer containing 1881 a.a. residues in total.
- Spike dimensions: 85 x 80 x 160 Å.



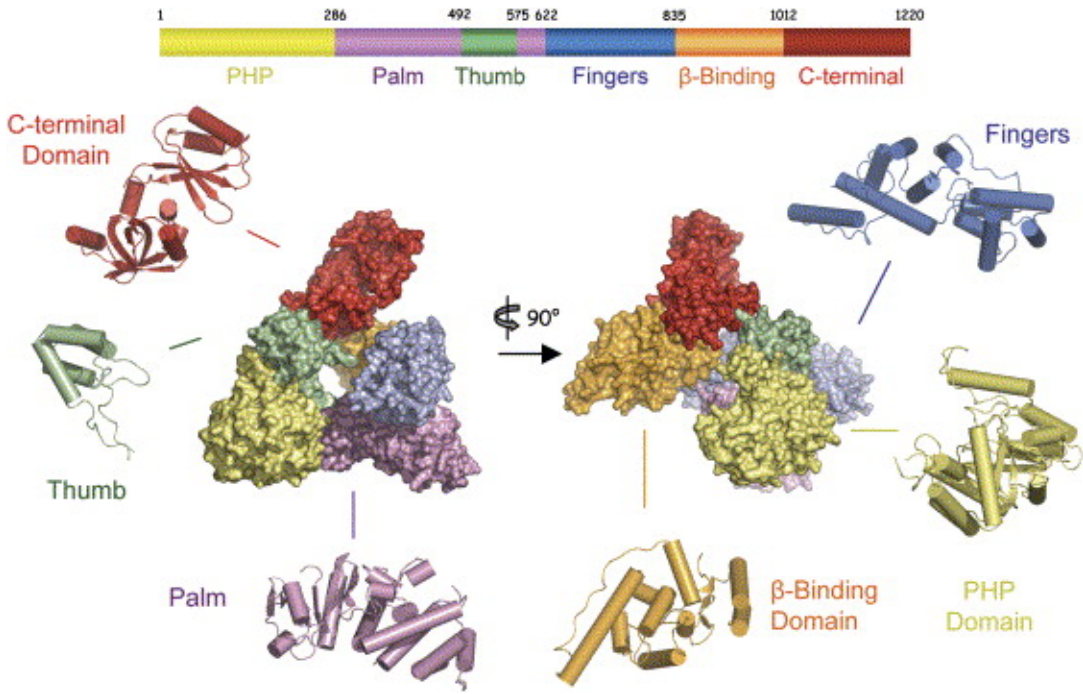
Protomer Trimer Surface of Trimer

Science Highlights

The Structure of *Thermus aquaticus* DNA Polymerase III at 3.0Å

S. Bailey, R. A. Wing and T.A. Steitz, Cell, 126, 2006, 893-904

- First eubacterial replicative DNA polymerase structure
- A monomer of ~140kDa made-up of six domains
- $C222_1$, *a*=175.1, *b*=186.9 and *c*=125.8Å



Taq DNA Polymerase III α subunit